Estradiol and its metabolites as novel biomarkers and targets in mesothelioma treatment

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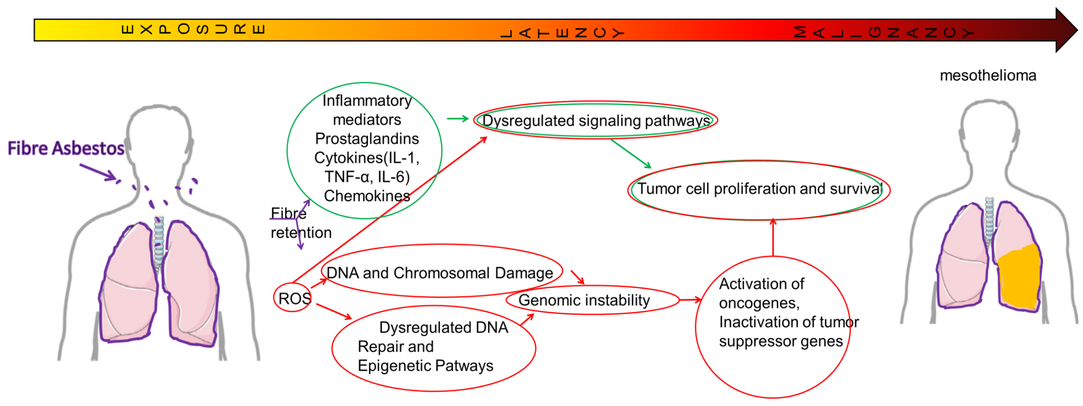
BACKGROUND

Malignant mesothelioma is an asbestos induced cancer that is difficult to diagnose and treat. It is often resistant to treatment and most patients survive less than a year (1,2). In this scenario, our previous studies explored the role of 17βestradiol in the pathogenesis of mesothelioma. In a group (n=29) of human mesothelioma samples the enzyme involved in the synthesis of 17βestradiol, the aromatase, was expressed as a cytoplasmic protein and its expression was significantly associated with poor survival of patients (3). Furthermore, we reported 17βestradiol staining in 95% of 57 human mesothelioma samples and its negative correlation trend toward the median post-diagnosis survival time. We observed in mesothelioma mouse xenografts, a correlated increase of tumour mass and serum 17βestradiol levels. Viceversa, a strong reduction of tumour mass and serum 17βestradiol levels was observed when the mice were treated with exemestane, an estrogen synthetase inhibitor(4-6). All this findings suggests to investigate the 17βestradiol and its related metabolites to uncover the mechanism involved in the pathogenesis of mesothelioma and to identify accurate biomarkers and targets for early diagnosis and treatment.



AIMS

- ✓ To evalute the role of 17βestradiol in the pathogenesis of mesothelioma .
- ✓ To evaluate the role of 17βestradiol and its metabolites as possible biomarker and target for early diagnosis and treatment.

17βESTRADIOL IS EXPRESSED IN MESOTHELIOMA SAMPLES WITH A PREDOMINANCE OF CYTOPLASMIC LOCALIZZATION AND TO A LESSER EXTENT NUCLEAR AND MEMBRANE. THE IRS OF 17βESTRADIOL IS MODERATE AND MILD POSITIVE IN MESOTHELIOMA SAMPLES WITH RESPECT TO STRONGLY POSITIVE OF MESOTHELIUM

A Characteristics of patients with meothelioma

Characteristics	Patients (n=60)
Gender	
Female	17 (28,3%)
Male	43 (71,7%)
Age (Years)	71,5±10 (48-89)
Histology subtypes	
Epithelioid	53 (88,33%)
Biphasic	2 (3,33%)
Sarcomatoid	5 (8,33%)
Exposure to asbestos	
Occupational	33 (55%)
Environmental	17 (28,3%)
Unknown	10 (16,7%)

B Immunohistochemical analysis of 17βestradiol and cellular localization

	Mesothelium (n=3)	IRS	Mesothelioma (n=60)	IRS
Cytoplasmic	3 (100%)	strongly positive	52(86,6%)	moderate
Nuclear			2 (3,3%)	mild
Cytoplasmic + Nuclear			3 (5%)	moderate
Cytoplasmic + Membrane			2 (3,3%)	mild
Cytoplasmic + Membrane + Nuclear			1 (1,7%)	moderate

IRS(Immunoreactive score) : 0-1 = negative, 2-3 = mild, 4-8 = moderate 9-12 = strongly positive

C

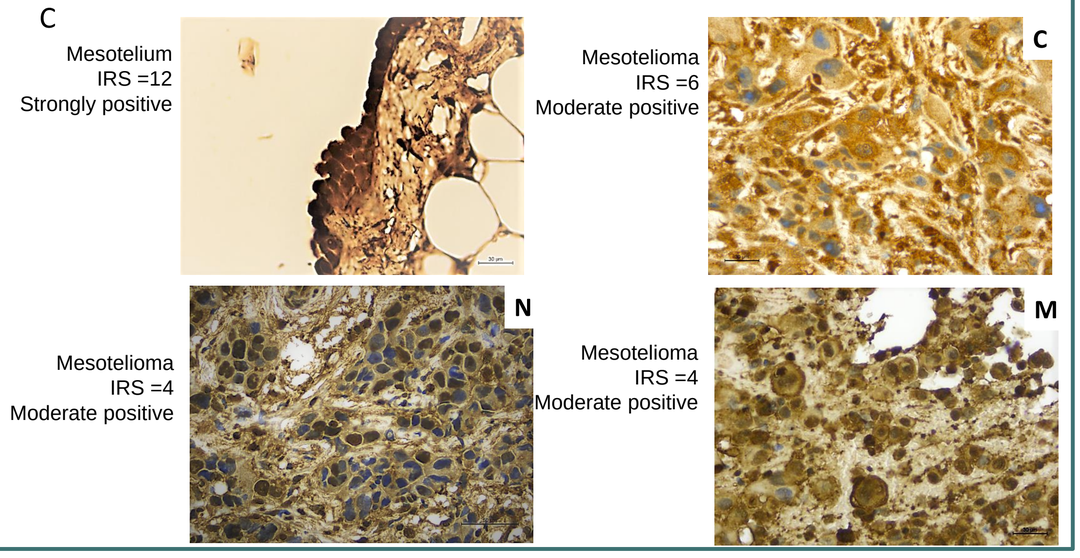


Figure 1. A Table of the characteristics of patients with mesothelioma from Mesothelioma biobank of Alessandria, **B** Table with cellular localizzation of 17βestradiol and immunoreactive score calculated as percentage of positive cells x intensity of staining, **C** Images representative of 17βestradiol staining in mesothelium and mesothelioma with the cytoplasmic (C) nuclear (N) and Membrane (M) localization.

SERUM 17βESTRADIOL LEVELS ARE HIGHER IN PATIENTS WITH MESOTHELIOMA THAN IN HEALTHY PEOPLE

A Analysis of 17βestradiol(E_2) in the serum .

		17βestradiol (pg/ml)
Healthy people	Female (n=2)	10 - 14
	Male (n=22)	10 - 42
Mesothelioma patients	Female (n=14)	12-157
	Male (n=33)	14-104

B

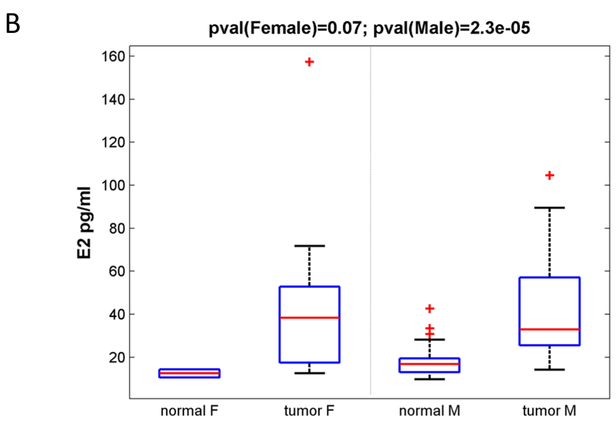


Figure 2. A Table of the levels of 17βestradiol (ELISA) in the popolations involved in the study. The lowest and highest levels of 17βestradiol for each individual group are shown. **B** The boxplot displays graphically groups of numerical data.

LONG-TERM 17βESTRADIOL PROMOTES PROLIFERATION OF NON MALIGNANT MESOTHELIUM (Met 5A) SPEROID CELLS IN PRESENCE OF EGF AND IGF

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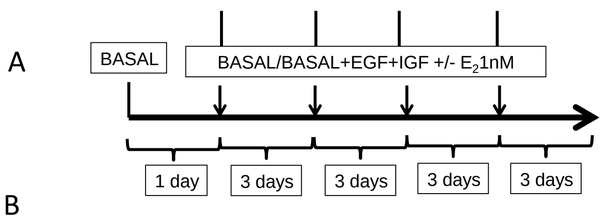

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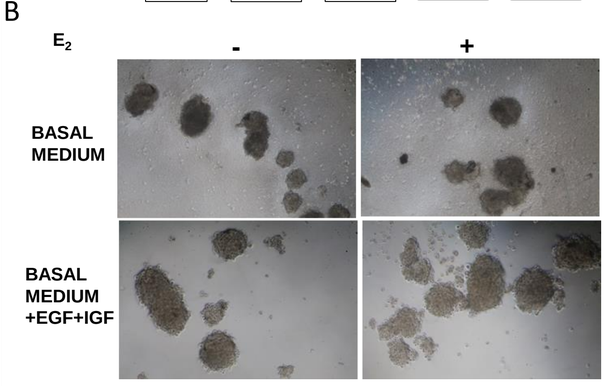

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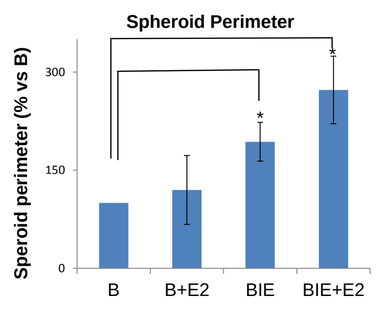

Figure 3. **A** Schedule of treatment for long time of non malignant mesotelium (Met 5A) spheroid cells with17βestradiol in basal medium and basal medium supplemented with EGF(5ng/ml) and IGF (1X). **B** Images representative of sphere formation after 13 days of treatment. **C** Quantification of spheroid perimeter by ImageJ. Results represent means ± SD.*P< 0.05

CONCLUSIONS

In patient with mesothelioma we observed that:

- **17βestradiol in mesothelioma tissues is localized with prevalence in the cytoplasm and to a lesser extent in the nucleus and membrane;**
- **17βestradiol in mesothelium tissues is localized in the cytoplasm with a strongly positive immunoreative score (IRS) than mesothelioma samples;**
- **The 17βestradiol levels in serum are higher in patient with mesothelioma with respect to healthy people;**
- **Because healthy people with normal 17βestradiol values possess strong expression in the mesothelium and mesothelioma patients with serum 17βestradiol levels higher than normal values possess lower expression of 17βestradiol in tumor tissue can be hypothesized that the progression of mesothelioma involves a release of 17βestradiol from the tumor to blood;**
- **Long-term 17βestradiol exposure not increase spheroid perimeter in non malignant mesothelium (Met 5A) spheroid culture. In the absense of EGF and Insulin, 17βestradiol by itself is unable to induce proliferation of non malignant mesothelium spheroid culture.**

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